Laboratory diagnosis of reproductive failure in beef cattle

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Abstract

Reproductive failure in beef cattle continues to be a source of significant economic loss for producers in all beef producing regions of the world. Reproductive failure includes everything from failure of conception associated with fertility problem in the dam or the sire all the way to full term stillborn or weak born calves. Abortion rates vary between herds, production systems and management styles, but in most situations, a rate much higher than 5% is usually deemed unacceptable and worthy of investigation. Abortion storms such as were historically seen with IBR could affect up to 40% of the pregnant animals. Today, abortion storms are rare, but losses that exceed 10% are still common. Implementing intervention strategies to impact ongoing abortion is usually limited, costly and often of questionable efficacy. Preventive programs may need modifications, but in reality, most producers already have basic vaccination programs in place. Diagnostic services for abortion disease diagnosis can vary greatly between laboratories, and associated costs can be significant. Therefore, it helpful for practitioners to understand the diagnostic process and inherent limitations of abortion diagnosis and to be able to help the producer determine if and when an investigation is warranted, and submit the appropriate samples to a laboratory that specializes in diagnosis of reproductive failure in livestock.

Key words: beef cattle, reproductive failure, abortion, diagnostics

Introduction

Substantial improvements in test development have given the diagnostician powerful tools for etiologic diagnosis. New multiplex polymerase chain reaction (PCR) formats are highly sensitive and allow rapid detection of multiple agents in a single test.[16] Immunocytochemistry and new bacterial ID systems are rapid and highly sensitive. Histopathology, routine culture, fungal culture, fluorescent antibody (FA) tests and virus isolation are still common and form the foundation of our approach to abortion diagnosis. Like all diagnostic laboratories, we are constantly re-evaluating old methods and evaluating new technologies for their ability to provide accurate diagnostic information. Remember, new technologies incur new costs that must eventually be passed on to the producer.

The development of new vaccines and vaccination strategies has reduced the impact of the once major reproductive infectious diseases such as IBR, BVD and leptospirosis.[8,9] However, these once major players are being replaced by an increase in opportunistic pathogens that appear to be emerging with changing production and management systems. In reality, every year significant financial losses due to reproductive failure still occur in spite of vaccine and management improvements. The practitioner is faced with the dilemma of trying to find answers for these losses where often none exists. The diagnostician is often faced with trying to make a definitive diagnosis where none is possible. The cycle tends to repeat itself every year during "abortion season".

Reproductive losses can occur at any stage of gestation. Embryonic mortality (up to 45 days) is often unnoticed and results in open animals or spread-out calving seasons. These early fetal losses are associated with a wide range of physiologic, nutritional, environment and non-infectious causes that often go unrecognized.[7] Infectious causes fetal loss during early gestation traditionally include *Tritrichomonas foetus*, *Leptospira borgpetersenii* serovar *hardjo* type Hardjobovis (Lepto hardjo) and bovine viral diarrheal virus (BVDV).[4] In most circumstances, embryonic loss occurs without recovery of a conceptus. Abortion implies expulsion of a fetus before full term and viability outside of the uterus. Stillbirth or premature delivery is expulsion of a term fetus that is considered viable. For these near-term fetuses, it is necessary to determine if the fetus was viable at expulsion or had been dead in utero. Antepartum death is characterized by variable degrees of autolysis, accumulations of blood-tinged fluids in body cavities, soft autolytic kidneys, and variable degrees of liquefaction of the brain. Tissues develop a uniform red brown appearance due to hemoglobin staining. Deaths associated with the parturition process are often less autolytic, display evidence of viability such as hemorrhage (functioning circulatory system), partial aeration of the lungs, meconium staining of the perineum and skin, swelling of the head and cervical region, subcutaneous edema and fractures of ribs and limbs associated with the fetal expulsion process. Animals that have survived the birth process and died shortly after will halve blood clots in umbilical vessels, aerated lungs and minimal free fluid in body cavities.

The routes by which infectious agents make their way to the fetus include hematogenous spread through the placental maternal interface where the placental chorioallantois is attached the lining of the uterus at the caruncle. Additionally, ascending infection from the vagina through the cervical os can also result in placental infection.[13] Infectious agent can colonize the placenta and penetrate into the amniotic fluid and is swallowed by the fetus. Fungal organisms can penetrate the placenta and result in colonization of the fetal skin. Hematogenous spread results in passage through the liver and to the remaining tissue through the vascular system. Fetal pneumonia in these cases results in interstitial accumulation of organism and inflammatory cells. For example, abortion associated with *Listeria monocytogenes* presents with massive bacterial growth with organisms present in blood vessels in most fetal tissues. With this organism, inflammation is generally mild compared to the massive number of organisms present in tissues. Organisms can also enter the lung through the airways by inhalation of infected amniotic fluid. This amniotic fluid will often contain clumps of meconium indicting advancing fetal stress due to hypoxia.

Fetal hypoxia can result from maternal hypoxia, maternal circulatory system failure or interference with oxygen transfer through the placental interface, most often associated with placentitis or premature placental separation. If possible, fetal compensatory mechanisms shunt blood to vital organs in an

attempt to maintain normal oxygen levels. Fetal respiration increases in an attempt to compensate for hypoxia. This labored breathing is often associated with the aspiration of amniotic fluid. If the placenta is compromised due to slow growing, opportunistic bacteria or fungi, and the fetus is not immediately overwhelmed by the infection, the slowly advancing placental damage will suffocate the fetus due to lack of oxygen or starve it for lack of nutrient transfer across the fetal maternal interface. If the fetus is not yet viable, abortion occurs; if the fetus is still viable but weakened due to hypoxia, low nutrient transfer and possible deleterious effect of chronic infection, the outcome is often a stillborn or weak-born calf.

Case submission

Appropriate collection and submission to samples for abortion diagnosis is critical for diagnostic success. Submitting a complete history is often the first critical component for diagnostic success. Include information about the herd history and details about the current abortion problem including an estimate on the gestational age. Table 1 contains information on the estimation of gestational age based of crown rump length, size and physical characteristics.[12] Information such as recent purchases or exposure to outside animals, illness in the dam or the herd and a history of vaccinations and treatments should be included. Additional information on nutritional management, feedstuffs and feeding practices is also useful when evaluating risks for feed associated opportunistic pathogens.

Collection and submission of appropriate samples that are not completely autolyzed should be the goal in every submission. Aborted fetuses are often retained in utero, macerated, mummified, severely autolytic, partially eaten, covered in mud and manure, buried in bedding, frozen solid or rotten from extreme heat. Superficial contamination can be rinsed away. Unfortunately, rotten is still rotten. Some samples are just unsuitable for evaluation. The intact fetus and complete placenta are ideal for submission if the laboratory is in close proximity to the producer. Gross examination and collection of tissue is conducted at the laboratory. Necropsy procedures can easily be performed in the field or clinic and involve exposure on the thoracic and abdominal cavities for collection of visceral tissues, removal of the head or brain and collection of appropriate tissues and body fluid as listed in Table 2. Tissues should be divided and portions submitted fresh (chilled or frozen is shipping delays are expected) and a portion submitted fixed in 10% buffered neutral formalin.

The placenta

The placenta is the most significant tissue for abortion diagnosis. If unavailable, the probability of diagnosis is significantly reduced. A whole, intact placenta is rarely received for examination. Often only a small portion of placenta is recovered and may be devoid of any cotyledonary structures. Histologic changes in placenta are often multifocal in distribution requiring multiple sections be examined to give the diagnostician the best chance of detecting subtle areas of placental damage. Placentitis results in disruption of placental functions including oxygen transport and exchange, nutritional support for the fetus, hormone and growth factor production that can affect normal parturition and fetal development. Chronic inflammation associated with release of cytokines and pro-inflammatory factors alter normal physiologic processes that occur at the fetal maternal interface. Fetal macrophages within the placenta are rare in the early gestational fetus, but by 8 months gestation, they have increased 10-fold. These macrophages are numerous within the allantoic stroma within areas of inflammation and often appear to contain debris or organisms in their cytoplasm. The role of these cells in cell defense against infectious agents as well as their role in dissemination of organisms is unknown.[14]

A complete placenta is a relatively large tissue and placental lesions are often focal to multifocal in distribution. Therefore, a single small section of placenta may miss significant changes and result in a missed diagnosis.

Maternal and fetal serology

Single serum samples from the dam are often submitted with abortion investigations, but they are usually of little value in abortion diagnosis. Positive serology for an individual animal at best indicates exposure to a specific agent or antigens to a specific agent in the form of vaccine. Separating the two responses is often impossible. Knowledge of vaccination history, types and brands of vaccine used and baseline serologic data from the specific laboratory performing the test is crucial to any serologic interpretation. Most of the opportunistic infections including environmental bacteria and fungi do not have validated serologic tests. Many infectious agents stimulate titer increases that predate expulsion of the fetus. Therefore, using paired serum samples on individual animals to detect changes in titers is also rarely useful for demonstrating evidence of specific abortion agents. A serologic profile comparing aborted

Table 1: Estimation of fetal gestational age

Crown-rump length (cm)	Age	Compartive size	Physical characteristics
1 cm	30 days		
10 cm	60 days	Mouse	
20 cm	90 days	Rat	
30 cm	120 days	Small cat	
45 cm	150 days	Large cat	
60 cm	180 days	Small dog	
80 cm	210 days	Large dog	Hair around eyes, tail, muzzle
100 cm	240 days		Hair on body, incisors visible
>100 cm	270 days		Near term, incisors erupted

Table 2: Samples submission for ruminant abortion diagnosis

Whole fetus and placenta
Submit whole fetus and placenta if proximity to laboratory is convenient, fresh (chilled) not frozen
If the entire fetus cannot be submitted, then the following tissues should be collected:

Fresh[1]		Formalin fixed[2]	
Lung (anterior lobes)	B,V	L:ung	Hp
Kidney	B (Lepto),V	Kidney	Hp
Liver	V	Liver	Hp
Spleen	V	Spleen	Hp
Heart	V	Heart	Hp
Placenta	B,V,M	Placenta	Hp
		Skeletal muscle	Hp
		Thymus	Hp
		Brain	Hp

Fetal stomach content BM Collect with sterile syringe and submit in snap cap tube

Fetal thoracic fluid/heart blood Collect with sterile syringe and submit in snap cap tube

Ocular fluid for nitrate/nitrite analysis Collect with sterile syringe and submit in snap cap tube

Maternal blood for serology[3]

Other: Feed and water samples

[1] Adequate fresh sample should be placed in leak- proof bags, chilled or frozen if delivery is delayed

[2] Fix in adequate (10x) volume 10% buffered neutral formalin, submit in leak-proof sealed container.

[3] Maternal blood can be collected and serum harvested and saved frozen for future use.

B-bacteriology, V-virology, M-mycology, Hp-histopathology

animals to normal controls is more often recommended. Serologic profiling on a significant number of animals in a herd may provide data on vaccination status for a given antigen as well as suspect exposure based on markedly elevated titers in the aborts versus the normal controls. Fetal serology is rarely used in routine diagnostics. If the fetus is old enough to be immunocompetent, fetal Immunoglobulin G (IgG) levels can be significantly elevated in fetal fluids in some infectious abortions. If IgG is elevated, then individual serologic tests can be performed as appropriate.[1] In *Neospora* caninum abortions, fetal and neonatal serology was used to detect in-utero infections in aborted fetuses or pre-colostral calves. Currently, we routinely bank fetal serum samples for testing if the need arises.

Bacterial infections

The vast majority of bacterial causes of abortion are opportunistic pathogens. These bacteria gain entrance to the blood stream of the dam and occasionally set up an infection in the placenta. *Trueperella pyogenes* and *Bacillus*, followed by *Escherichia coli*, Histophilus somni, *Pasteurella*, *Listeria*, *Staphylococcus*, *Streptococcus* and basically any other bacteria that can find its way into the blood stream, can be opportunistic pathogens. These opportunists are usually associated with sporadic late term abortions, unless specific risk factors give a particular organism the chance to affect multiple animals. Cattle with abscesses or a history of feet problems seem to have problems with *Trueperella pyogenes*. Cattle exposed to processed bales with a great deal of soil-associated spoilage can have increased problems with *Bacillus sp. Listeria sp*. is usually associated with poorly fermented silage feeding. Gross lesions are rare, but can include exudate on the placenta surface, or possibly increased fluid in body cavities, occasionally with fibrin. Histologic lesions include suppurative fetal pneumonia, mild perivascular inflammation in the epicardium and to a lesser extent, myocardium, increased portal inflammatory cells in the liver, and inflammatory cell pooling in blood vessels in the brain and other tissues. A variably severe, multifocal, necrotizing and suppurative placentitis is a common lesion if adequate placenta is examined. Numerous intralesional bacteria are often observed histologically, especially in the case of *Trueperella pyogenes*, *Campylobacter* and *Salmonella* induced abortion.

Multiple species, serovars and types of *Leptospira* including *hardjo* type harjobovis, *pomona*, *icterohaemorrhagiae, grippotyphosa* and most likely many others can be involved in bovine embryonic loss and abortion.[5] Specific gross and histologic lesions have been described historically, but Leptospira abortions are so infrequent today, that I suspect that most diagnosticians would not recognize them. In the upper Midwest, the near universal use of multivalent vaccines for *Leptospira* have significantly reduced its diagnosis associated with abortion in cattle. Culture of this organism is not practical due to time and cost constraints. Microscopic detection by dark-field examination of fetal fluids or silver-stained histologic sections is occasionally used, although the sensitivity of the techniques is low. A common technique, fluorescent antibody (FA) staining of kidney homogenates with multivalent antisera, is frequently used.

Again, the sensitivity may be low, especially with host adapted *Leptospira* such as type hardjobovis. New PCR tests are currently in use, and have the benefit of speed, specificity and sensitivity. The PCR format is routinely used to detect carrier cows that shed the harjobovis organism in urine and is becoming more common for detection of *Leptospira* organisms in abortions.

Viral infections

Viral causes of abortion include bovine herpesvirus type I, the cause of infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), and to a lesser extent, bovine herpes type 4 (BHV-4). Numerous references are available that describe these agents in detail.[3,4,6,9] IBR associated abortions have decreased dramatically since the introduction of effective vaccines. Recently, increased IBR abortions have been reported in unvaccinated or questionably vaccinated cows exposed to modified live vaccine during gestation. Gross and histologic lesions are commonly observed with IBR and can include pale foci in the liver that corresponds with the multifocal necrotizing lesions that are present in several fetal tissues including liver, lung and spleen. Similarly, the incidence of BVDV has also decreased in the last several years, most likely associated with increased vaccination. BHV-4 is considered an opportunistic viral pathogen and its role in abortion is difficult to determine. Diagnostic procedures for viral abortion agents vary between laboratories. Fluorescent antibody tests are rapid and usually of acceptable sensitivity. Virus isolation is considered a tried-and-true method, especially for discovery of new agents, but it is very expensive, time-consuming and requires technical expertise. The advantage of virus isolation is that you have an isolate for further study or vaccine production at the end of the procedure. PCR-based tests have replaced other techniques in many laboratories due to their speed, specificity and sensitivity. Multiplex PCR is currently available for IBR and BVD.

Mycotic infections

Mycotic abortion is common worldwide. The common agents include *Aspergillus fumigatus, Aspergillus, Candida* and a variety of environmental species.[10,11] These organisms are ubiquitous saprophytes in the environment and often increase in numbers in moldy feedstuffs or bedding. Abortions usually occur when cattle a fed stored or processed feedstuffs. The conidia from these organisms enter the respiratory tract or digestive tract and gain entrance into the bloodstream and spread to the uterus and placenta. Gross lesions include thickening and roughening of cotyledons and intercotyledonary spaces. Lesions are often localized and may not be present if only a small portion of placenta is submitted. Histologic lesions if present will confirm a severe necrosuppurative placentitis and stromal arterial vasculitis. Fungal hyphae are often associated with these necrotic lesions.

Diagnosis of mycotic abortion can be accomplished by fluorescent KOH procedures on placental scrapings that allow visualization of fungal elements. Histologic identification of fungal elements is also useful using special histochemical stains. Culture of fungal organisms from stomach content and placenta requires special media with added antibiotics to suppress bacterial growth. When a mixed growth of fungal organisms is isolated, the significance of the results should be questioned, but not dismissed. Multiple fungal species are often present in feedstuffs, so dual infections cannot be completely eliminated as a possible diagnosis. If any particular fungal organism is isolated in heavy growth, or isolated in heavy growth from fetal stomach content and placenta, and compatible placental lesions exist, then causality should be considered.

Protozoa

Protozoal agents associate with abortion include *Neospora caninum* and *Tritrichomonas fetus*.[2] *Neospora caninum* is vertically transmitted from dam to congenitally infected normal offspring as well as horizontal transmission by ingestion of infective oocysts shed by the canine definitive host. Epidemic abortions were more common historically when most cattle were naïve to infection. The most common presentation with *Neospora caninum* today is sporadic or endemic abortions. The dam is clinically normal and most abortions occur between 5 and 7-months gestation. Compatible lesions include multifocal necrosis and gliosis and non-suppurative epicarditis, myocarditis and myositis. Occasionally, similar focal lesions are present in other tissues. Immunohistochemistry is used for detection of the organism in context of the histologic lesion. PCR and a number of serologic tests are also available for diagnosis. Caution should be used if lesions are very mild or non-typical as most calves born to seropositive dams will be congenitally infected, and the abortion could have been caused by other agents.

Tritrichomonas foetus is most often associated with early embryonic death and early abortions in cattle. The organism can be cultured in special media from carrier bulls and occasionally from infected cows or recovered fetuses. PCR techniques have also been developed. In aborted fetuses, a mixed pneumonia is present and occasional protozoa compatible with *Tritrichomonas foetus* can be found.

Non-infectious abortion

Non-infectious causes of abortion are often lumped together and include the variety of genetic, nutritional and environmental factors associated with reproductive failure. This category is often a catchall and is often overlook in most diagnostic scenarios. Genetic causes of early embryonic mortality often go unnoticed. Embryonic loss associated with chromosomal defects or lethal mutations are rarely ever detected. Obvious congenital anomalies that present at birth often fit in one of two categories. The first case is those with established genetic conditions, often with known genetic defects and testing strategies to eliminate the trait from the breed. The second and most common is everything else with a congenital anomaly. Caution is warranted in using the word genetic too early when investigating congenital malformations.[15] Many of the animals involved in these situations are extremely valuable and data must be collected carefully and thoroughly before reaching any conclusion. The majority on non-genetic causes are probably still unknown, but we recognize that nutritional factors, toxic plants, chemical exposure and viruses as possible suspects to be considered. Nutritional factors, including trace mineral, vitamins, protein and energy, can contribute to increase fetal loss and poor postnatal survival. Many of these causal links are difficult to prove, however, the possibility of nutritional component should be considered, if for no other reason than to gives the producer the opportunity to evaluate and correct nutritional problems before they get worse.

Summary

Successful abortion diagnosis in beef cattle requires input from the producer, practitioner and diagnostician. Unfortunately, in spite of everyone's best efforts, many investigations still result in a diagnosis of idiopathic abortion. However, if this diagnosis is made after a complete and systematic investigation, on appropriate and reasonably preserved samples, then I take comfort that we did our best as a practitioner and diagnostician working for the benefit of the producer.

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